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PRECLINICAL BLOOD CHEMISTRY SAFETY PROFILE STUDIES OF "ROHITAKARISTA" ON THE KIDNEY FUNCTION AFTER CHRONIC ADMINISTRATION TO MALE SPRAGUE-DAWLEY RATS

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ABSTRACT

Rohitakarista (RHT) is an Ayurvedic preparation used as a traditional medicine in the treatment of splenomegaly. To find out the effect of chronic administration of RHT on serum blood chemistry profile, it was administered chronically to the male Sprague-Dawley rats at a dose of 40 ml/kg for 28 days. In this study, the albumin content was decreased (9.17 %) in RHT treated male rats and it was statistically significant (p=0.047), the globulin content was highly significantly (p=0.012) increased (53.10 %) as a result the decrease (43.18%) in the Albumin / Globulin (A/G) ratio was statistically highly significantly different from their corresponding control values (p=0.005). There were a statistically very highly significant (p=0.001) decrease of blood urea nitrogen (BUN) level (23.97%) and BUN/Creatinine ratio (21.62 %). It was observed that there was a 15.0% decrease in serum uric acid content of RHT treated male rats in comparison to their control male rats which was also statistically significant (p=0.049).

Keywords: Rohitakarista, Blood urea nitrogen, Albumin, Creatinine, Ayurvedic formulation.

INTRODUCTION

Ayurvedic medicines are somewhat inexpensive and have wide acceptability among the general populace, particularly in rural areas of the country by using Ayurvedic medicine costly and extensive procedures of clinical investigations can be avoided in many cases [1]. They have a good safety profile also [2]. People in these selected areas have the choice to get treatment at a cheaper price depending on their choice. Many herbal medicines here now have reputation as good and efficacious remedies for a number of diseases [3]. Currently, the World Health Organization (WHO) has officially recognized and recommended large-scale use of herbal (Unani and Ayurvedic) medicines, particularly in the developing countries, as an alternative system of medicine to provide health care services at the primary health care level [4]. An estimated 1.5 billion people of the world's population, according to WHO, are now getting treatment with these medicines [5, 6].

Rohitakarista (RHT) is an Ayurvedic preparation used as a traditional medicine in the treatment of splenomegaly in the rural population [7]. An animal model is suitable for predicting what may happen in a small percentage of humans and it is usual to carry out serum blood chemistry profile investigations in animals in the course of developing any new medical product [8, 9].

Rohitakarista is included (page 125) in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (Approved by the Government of

Bangladesh vide Ministry of Health and Family Welfare Memo No. Health-1/Unani-2/89/ (Part-1) 116 dated 3-6-1991). Bangladesh National Formulary of Ayurvedic Medicine is compiled by the National Unani and Ayurvedic Formulary Committee and published by the Bangladesh Board of Unani and Ayurvedic Systems of Medicine, 38, Bangabandhu Avenue, Dhaka-1000 under the authority vested in the Board vide section 13(j) of the Bangladesh Unani and Ayurvedic practitioners Ordinance, 1983 in collaboration with the World Health Organization [10]. Permission to manufacture at industrial scale is printed in page no. 535 (column 2: Product code 16.60). Directorate of Drug Administration has issued Notification DA/Admin/1-10/96/6212 dated 19th October 1996 has issued license under Drug Act, 1940 and Rules there under and Drug (Control) Ordinance 1982 for local manufacture and sale in Bangladesh. (Published Bangladesh Gazette #24 Part VI dated Thursday, June 11th 1998.) At present a good number of Ayurvedic manufacturers are manufacturing and marketing the Classical Ayurvedic Medicinal Preparation [11-17].

Rohitakarista (RHT) is widely used in the treatment of diseases of spleen, abdomen, abdominal swelling or tumor, mal-absorption syndrome or sprue and enlarged prostate [18-23]. This research work on Ayurvedic formulation, Rohitakarista unfolds a field of its toxicological aspects by utilizing experimental animals (Rats). That is why; we designed our current experiment to observe the effect of chronic administration of RHT to Sprague-Dawley rats at a high dose. The objective is to have a better understanding of the potential toxicological profile of the drug under study and to decide how justifiable the use of this drug is under the stated conditions. The study provides directions for further research as well. The research work has been carried out in order to characterize the kidney function profile of the Ayurvedic medicinal preparation, RHT.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents: For the toxicological study, Rohitakarista (RHT) was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was purchased from ACI Pharmaceuticals Limited, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Human GmbH, Wiesbaden, Germany.

Experimental Animals: Six to eight-week old male Sprague-Dawley rats bred and maintained at the animal house of the Department of Pharmacy, Jahangirnagar University, were used in the toxicological experiment. These animals were apparently healthy and weighed 60-70 g. The animals were housed in a well-ventilated clean experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. They were fed with rat chow prepared according to the formula developed at Bangladesh Council of Scientific and Industrial Research (BCSIR). Water was provided *ad libitum* and the animals maintained at 12 h day and 12 h night cycle. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals approved by Ethical Review Committee, Faculty of Life Sciences, Department of Pharmacy, Jahangirnagar University.

Experimental Design

Chronic toxicity studies: Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with RHT and another was used as a control. The control animals were administered with distilled water only as per the same volume as the drug treated group for 28 days. For all the pharmacological studies the drugs were administered per oral route at a dose of 40 ml/Kg body weight [24]. After acclimatization, Ayurvedic medicinal preparation was administered to the rats by intra-gastric syringe between the 10 am to 12 am daily throughout the study period. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. The experiment animals were marked carefully on the tail which helped to identify a particular animal. By using identification mark, responses were noted separately for a particular period prior to and after the administration [25].

Blood Samples Collection and Preparation of Serum: At the end of the 28 days treatment period, after 18 h fasting, rats from each group were anaesthetized by administration (i.p) of ketamine (500 mg/kg body weight) [26]. Blood samples were collected from post vena cava of rats into plain sample tubes for serum generation for biochemical analysis. Serum was obtained after allowing blood to coagulate for 30 minutes and centrifuged at 4000 rpm for 10 min using bench top centrifuge (MSE Minor, England). The supernatant serum samples were collected using dry Pasteur pipette and stored in the refrigerator for further analysis. All analyses were completed within 12 hours of sample collection [27].

Determination of Biochemical Parameters: Biochemical analysis was carried out on serum to assess the state of the liver [28] and kidney [29].

Biochemical studies involved analysis of parameters such as Total Protein [30], Albumin by Bromacresol green method [31], Creatinine [32], Blood Urea Nitrogen (BUN) [33] and Uric Acid [34]. The absorbances of all the tests were determined using spectrophotometer (UV-Visible Spectrophotometer Model No. UV-1601 PC).

Statistical Analysis: The data were analyzed using independent sample t-test with the help of SPSS (Statistical Package for Social Science) Statistics 11.5 package (SPSS Inc., Chicago Ill). All values are expressed as mean ± SEM (Standard error of the mean) and $p<0.05$, $p<0.01$, $p<0.001$ was taken as the level of significance.

RESULTS AND DISCUSSION

Daily oral dose of RHT (40 ml/Kg) did not cause any physical abnormalities or death after four week treatment period. In a previous study, Hasan et al (2014) performed the acute toxicity of this drug. Administration of doses up to 80 ml/Kg (the highest dose) produced no mortality of animals which was accompanied by normal physical activity of the tested animals [35]. This study strengthens the safety of the drug.

Effect of RHT on Total Serum Protein, Albumin, Globulin content and A/G ratio in male rats: After 28 days of chronic administration of the RHT preparation the total protein, albumin content and the calculated ratio of albumin to globulins, termed the A/G ratio in serum were determined in the male rats.

Proteins are important parts of all cells and tissues. The total protein test measures the total amount of two classes of proteins found in the fluid portion of blood: albumin and globulin. Albumin helps prevent fluid from leaking out of blood vessels and globulins are an important part of immune system [36, 37].

Normal range of total protein content: 6-8 g/dl. In the study, the total protein content in the serum was increased (10.76 % incr.) in the RHT treated male rats. The increase in total protein was though not statistically significant yet it was noticeable ($p=0.108$) (Table-2). Drugs that can increase total protein measurements include anabolic steroids, androgens, corticosteroids, dextran, growth hormone, insulin, phenazopyridine, and progesterone [38]. The increase of the total protein in the RHT treated experimental population can be due to chronic inflammation, adrenal cortical hypofunction, liver dysfunction, hypersensitivity states.

Serum albumin test can help to determine if a patient has liver disease or kidney disease, or if the body is not absorbing enough protein. In the study, on the contrary to the findings regarding serum total protein content, the albumin content was decreased (9.17 % decrease; $p=0.047$) in RHT treated male rats and the decrease was statistically significant (Table-2). Serum albumin level decreased due to liver dysfunction, malnutrition and mal-absorption, hypothyroidism, nephrotic syndrome due to kidney disease, protein losing-enteropathy (protein is lost from the gastrointestinal tract during diarrhea), chronic illness, Chronic inflammatory diseases, inflammation, Insufficient anabolic hormones such as Growth Hormone, DHEA and testosterone [38]. The decrease of albumin in the RHT treated experimental population can be due to any of the factors mentioned above.

Globulins are the key building block of antibodies. Globulins include gamma globulins (antibodies), beta globulins, alpha-2 globulins, and alpha-1 globulins and a variety of enzymes and carrier or transport proteins. Since the gamma fraction usually makes up the largest portion of the globulins, antibody deficiency should always come to mind when the globulin level is low [38]. In the study, the globulin content was highly significantly (53.10 % increase; $p=0.012$) increased (Table-2). Chronic infections, liver disease (biliary cirrhosis), fatty necrotic liver, kidney dysfunction (Nephrosis), ulcerative colitis, rheumatoid arthritis, leukemia, multiple myelomas, increased amount of nonspecific protein, and autoimmune disorders such as collagen diseases can affect globulin level. The increase of globulin in the RHT treated experimental population can be due to any of the factors mentioned above.

The liver can function adequately on 20% of liver tissue, thus early diagnosis by lab methods is difficult. A reversed A/G ratio may be a helpful indicator. Normally this ratio exceeds 1.0 but in disease conditions which selectively affect albumin levels, are associated with lesser ratios [38]. In the study, the decrease (43.18% decrease; $p=0.005$) in the Albumin / Globulin ratio was statistically highly significantly different from their corresponding control values (Table-2). A low A/G ratio may reflect overproduction of globulins such as seen in multiple myeloma or autoimmune diseases or underproduction of albumin such as may occur with cirrhosis or selective loss of albumin from the circulation as may occur with kidney disease (nephrotic syndrome), liver dysfunction. The decrease of Albumin/Globulin ratio (A/G ratio) in the RHT treated experimental

population can be due to any of the factors mentioned above.

Effect of RHT on Creatinine, BUN, Urea, Uric Acid level in male rats: Kidney function test was performed to measure the creatinine and urea content in the serum. These two contents can provide information about how effective the kidney function is? Creatinine is a breakdown product of creatine, which is an important part of muscle. The laboratory test is performed to measure the amount of creatinine in the blood. The test is done to evaluate kidney function. If kidney function is abnormal, creatinine levels will increase in the blood [39-45]. There was a statistically insignificant decrease in the creatinine (2.90% decrease; p=0.622) content in serum in the RHT treated male rats (Table-3). Lower than normal level of creatinine reveal lack of nephro-toxicity [46-51].

BUN stands for blood urea nitrogen. Urea nitrogen is what forms when protein breaks down. A test can be done to measure the amount of urea nitrogen in the blood. The BUN test is often done to check kidney function. BUN Increases by 10-20 mg/dl/day if renal function absent. Serum creatinine is a better measure of renal function and BUN is reabsorbed at renal tubules [52-54]. Similar to creatinine, a statistically very highly significant (p=0.001) decrease of blood urea nitrogen (BUN) level (23.97% decrease) in the serum was noted in comparison to their control group (Table-3). Decrease of BUN level may be seen in severe liver disease, malnutrition, and sometimes when a person is over-hydrated. A decrease of BUN level may indicate lower risk of kidney disease.
BUN-to-creatinine ratio is considered a reliable test that helps in detecting kidney problems. BUN and creatinine are two compounds found in the blood and the amount of these substances is directly governed by the functioning of the kidneys. The principle behind this ratio is the fact that both urea (BUN) and creatinine are freely filtered by the glomerulus, however urea reabsorbed by the tubules can be regulated (increased or decreased) whereas creatinine reabsorption remains the same (minimal reabsorption) [52-54]. Any dysfunction of the kidneys can increase or decrease the quantity of these compounds in the blood. In the study, the decrease in BUN/Creatinine ratio (21.62 % decr.) was statistically very highly significant (p=0.001) (Table-3). There are various factors that affect the BUN/Creatinine ratio. The causes of low BUN/Creatinine ratio are muscle injury, syndrome of inappropriate antidiuretic hormone secretion (SIADH), cirrhosis. ADH (vasopressin) is produced in the hypothalamus and stored in the posterior pituitary. When released, it increases water reabsorption by the kidneys, thus increasing the fluid content of the body and decreasing BUN/Creatinine ratio. RHT should be used with caution in those individuals who are prone to edemic congestion or other ill effects as a consequence to increase in the fluid content of the body.

Uric acid is a chemical created when the body breaks down substances called purines, which are nitrogen-containing compounds found in the body in substances such as DNA. Most uric acid is removed from the body by the kidneys and is excreted in the urine; the remainder is eliminated in the stool. If too much uric acid is produced or not enough is excreted, it can accumulate in the body and cause increased levels in the blood (hyperuricemia). The accumulation of too much uric acid is due to either increased production, decreased elimination, or some combination of both [55, 56]. A 15.0 % decrease in serum uric acid content of RHT treated male rats in comparison to their control male rats was observed which was statistically significant (p=0.049). If less uric acid is produced or the amount produced is totally excreted in a good amount, it reveals a decrease in the uric acid level in the blood. Low levels of uric acid in the blood, as in this study, are seen much less commonly than high levels and are seldom considered cause for concern [52-54].

CONCLUSION

From the above data it can be concluded that RHT should not be administered chronically at a higher dose as it may cause liver disease. Further studies should be done by reducing the administered dose. Thus RHT is to be taken under medical supervision only at a dosage of 12–24 ml once or twice a day usually advised after food. If needed, it can be mixed with equal quantity of water.

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Table 1: Name of the ingredients/herbs used in the preparation of Rohitakarista

Name of Plants / Ingredients	Botanical Name	Used Parts	Family	Amount Used
Rohitaka	*Tecomella undulata*	Stem & Bark	Bignoniacea	4.800 kg
Water for decoction				49.152 L
	reduced to			12.288 L
Guda (Molasses)				9.600 kg
Amalaki	*Embelica officinalis*	Fruit powder	Euphorbiaceae	48 g
Bibhitaka	*Terminalia beleracia*	Fruit powder	Combretaceae	48 g
Cavya	*Pipper retrofractum*	Stem	Piperaceae	48 g
Citraka	*Plumbago zeylanica*	Root	Plumbaginaceae	48 g
Dhataki	*Woodfordia fruticosa*	Flower	Lythraceae	768 g
Ela	*Elettaria cardamomum*	Sod	Scitaminaceae	48 g
Haritaki	*Terminalia chebula*	Fruit powder	Combretaceae	48 g
Pippali	*Pipper longum*	Fruit	Piperaceae	48 g
Pippali mula	*Pipper longum*	Root	Piperaceae	48 g
Rohitaka patra	*Tecomella undulata*	Leaf	Bignoriacea	48 g
Sunthi	*Zingiber officinale*	Rhizome	Zingiberaceae	48 g
Tvak	*Cinnamomum zeylanicum*	Stem & Bark	Lauraceae	48 g

Table 2: Effect of Rohitakarista (RHT) on Total Serum Protein, Albumin, Globulin content and A/G ratio in male rats.

Parameters	Control	RHT	p values	% Change
Total Protein (TP)	44.13 ± 2.16	48.88 ± 0.74	0.108	↑10.76 %
Albumin	30.00 ± 1.07	27.25 ± 0.67	0.047	↓9.17 %
Globulin	14.13 ± 1.73	21.63 ±1.93	0.012	↑53.10 %
A/G ratio	2.34 ± 0.28	1.33 ± 0.12	0.005	↓43.18 %

Table 3: Effect of Rohitakarista (RHT) on Creatinine, BUN, BUN/Creatinine ratio, Uric Acid level in male rats

Parameters	Control	RHT	p values	% Change
Creatinine	0.86 ± 0.04	0.84 ± 0.03	0.622	↓2.90 %
Blood Urea Nitrogen (BUN)	24.86±0.13	18.90±0.11	0.001	↓23.97%
BUN/Creatinine	29.14±0.44	22.84±0.33	0.001	↓21.62%
Uric Acid	1.75 ± 0.09	1.49 ± 0.09	0.049	↓15.00 %

REFERENCES

1. Zhang X. Traditional medicine and alternative medicine in the world an overview. In: The International Symposium on Herbal Medicine, King Fahad Hospital, Jeddah, Saudi Arabia: 1997, pp. 30.
2. Ernst E. Pharmacoepidemiol Drug Saf, 2002; 11(6): 455-6
3. WHO. Regional Office for the Western Pacific Seminar on the Use of Medicinal Plants in Health Care, Final Report, Tokyo, Japan: 1977, pp. 13–7.
4. WHO. WHO Launches the First Global Strategy on Traditional and Alternative Medicine, Press Release WHO/38: 2002.
5. WHO. Consultation Meeting on Traditional Medicine and Modern Medicine: Harmonizing the Two Approaches. Geneva, World Health Organization, (document reference (WP) TM/ICP/TM/001/RB/98–RS/99/GE/32(CHN): 1999a.
6. WHO. Traditional, Complementary and Alternative Medicines and Therapies. Washington DC, WHO Regional Office for the Americas/Pan American Health Organization (Working group OPS/OMS): 1999b.
7. Khan MR. Sicknesses, Diseases, Treatments and Medical Costs by Socioeconomic Variables in Bangladesh. Bangladesh Institute of Development Studies, Dhaka: 1994.

8. Akerele O. Fitoterapia, 1992; 63: 99-110.
9. WHO Scientific Group. Principles for Preclinical Testing of Drugs Safety, Technical Report Series, World Health Organization, Geneva: 1967; 341: 9–11.
10. Anonymous. Bangladesh National Formulary of Ayurvedic Medicine Approved by the Government of Bangladesh vide Ministry of Health and Family Welfare Memo No. Health-1/Unani-2/89/ (Part-1) 116 dated 3-6-1991). National Unani and Ayurvedic Formulary Committee Bangladesh Board of Unani and Ayurvedic Systems of Medicine, 38, Dhaka-1000, Bangladesh: 2011b.
11. Anonymous. Vaidya Yoga Ratnavali (Formulary of Ayurvedic medicines). IMPCOPS, (The Indian Medical Practitioner's Co-Operative Pharmacy & Stores Ltd), Madras, India: 1968, pp. 592.
12. Anonymous. Ayurvedic Formulary of India, The Government of India, Ministry of Health and Family Welfare, Department of Health, Volume I, Part I, first edition, XXXVI, New Delhi, India: 1978a, pp. 324.
13. Anonymous. Hand book of Ayurvedic and herbal medicines with formulae: with directory of manufacturers and suppliers of plants, equipment and machineries, packaging materials and raw materials suppliers. Engineers India Research Institute, Delhi, India: 1978b, pp. 382.
14. Anonymous. Handbook of Domestic Medicine and Common Ayurvedic Remedies. Central Council for Research in Ayurveda and Siddha, New Delhi, India: 2005, pp. 538.
15. Anonymous. Classical Ayurvedic Prescriptions for Common Diseases. Central Council for Research in Ayurveda and Siddha. Department of AYUSH, Ministry of Health & Family Welfare Government of India, New Delhi, India: 2010, pp. 149.
16. Anonymous. Ayurvedic Formulary of India. The Government of India, Vol. I, part 3, LXXVI, New Delhi, India: 2011a, pp. 710.
17. Pandey G. BhaisajyaRatnavali, text with English commentary and supplements. Banaras Ayurveda Series 8, vol. 1, XXVIII. Varanasi, India: 2005, pp.740.
18. Anonymous. Treatment Guideline for Ayurvedic Medicine, Department of Homeo and Traditional Medicine, DGHS, Government of the People's Republic of Bangladesh. Dhaka, Bangladesh: 2006, pp. 70.
19. Dash B. Diagnosis and Treatment of diseases in Ayurveda, Vol I-V. Concept Publishing Company, New Delhi, India: 1984, pp. 2578.
20. Dastur JF. Everybody's guide to Ayurvedic medicine-A repertory of therapeutic prescriptions based on the indigenous system of India. Taraporevala Sons and Co., Bombay, India: 1960, pp. 212.
21. Mishra, Chandra L. Scientific Basis for Ayurvedic Therapies. CRC Press: 2010, pp. 626.
22. Nadkarni AK. Indian Materia Medica, Vol. 1. Popular Book Depot, Bombay, India: 1976.
23. Verma HK. Comprehensive Book of Ayurvedic Medicine for General Practitioners with Annonated Key References Vol I. Kalyani Publishers. New Delhi, India: 1991, pp. 196.
24. Gad SC. Intl J Tox, 1988; 7(2): 127-38.
25. Stevens KR, Gallo MA. Practical consideration in the conduct of chronic toxicity studies, Principles and Methods of Toxicology, 2nd edn. Chap. VIII: 1989.
26. Ringler H, Dabich L. Hematology and clinical biochemistry. In: The Laboratory Rat Biology and Disease [Baker HL ed]. American College of Laboratory Animal Medicine Series Academic Press: 1979.
27. Wolford ST, Schoer RA, Gohs FX, Gallo PP. J Tox Environ Hlth, 1986; 18: 161-88.
28. Pratt DS. Liver chemistry and function tests. In: Feldman M, Friedman LS, Brandt LJ, eds. Sleisenger and Fordtran's Gastrointestinal and Liver Disease. 9th ed., Saunders Elsevier; chap 73, Philadelphia: 2010.
29. Clarkson MR, Friedewald JJ, Eustace JA, Rabb H. Acute kidney injury. In: Brenner BM, eds. Brenner and Rector's The Kidney. 8th ed., Pa: Saunders Elsevier; chap. 29, Philadelphia: 2008.
30. Doumas BT. Clin chem, 1975; 21: 1159-66.
31. Doumas BT, Watson WA, Biggs HG. Clin Chim Acta, 1971; 31: 87-96.
32. Bartels H, Böhmer M. Clin Chim Acta, 1971; 32(1): 81-5.
33. Tabacco A, Meiattini F, Moda E, Tarli P. Clin Chem, 1979; 25(2): 336-7.
34. Fossati P, Prencipe L, Berti G. Clin Chem, 1980; 26(2): 227-31.
35. Niyati KNR, Hasan MR, Lopa SS, Chowdhury IA, Khalil M, Binoy MH, et al. Int J Pharm, 2014; 4(4): 168-74
36. Alper CA. N. Eng. J. Med, 1974; 291: 287-90.
37. Naganna B. Plasma proteins. In: Textbook of Biochemistry and Human Biology, 2nd edition. Talwar GP, Srivastava LM and Moudgil KD. Prentice- Hall of India Private Ltd., New- Delhi, India: 1989, pp. 59-61.
38. Pagana KD, Pagana TJ. Mosby's Manual of Diagnostic and Laboratory Tests. In Chapter 2, Blood Studies. 4th Edition, Elsevier Health Sciences: 2009, pp. 442-5.
39. Bovee KC. Toxicol Pathol, 1986; 14: 26.
40. Kluwe WM. Toxicol Appl Pharmacol, 1981; 57: 414-24.

41. Loeb WF. Toxicol Pathol, 1998; 26: 26-8.
42. Mitchell FL, Veall N, Watts RWE. Ann. Clin. Biochem, 1972; 9: 1-20.
43. Price RG, et al. Hum Exp Toxicol, 1996; 15 (suppl. 1): 10–19.
44. Price RG. Comp Clin Path, 2002; 11: 2-7.
45. Zalups RK, Lash LH. Methods in renal toxicology. Boca Raton, FL, CRC Press: 1996.
46. Fent K, Mayer E, Zbinden G. Arch Toxicol, 1988; 61: 349-58.
47. Gray JE. CRC Crit Rev Toxicol, 1977; 5: 115-44.
48. Lauwerys R, Bernard A. Toxicol Lett, 1989; 46: 13-29.
49. Palm M, Lundblad A. Vet Clin Pathol, 2005; 34: 232-6.
50. Stonard MD. J Appl Toxicol, 1990; 10: 267-74.
51. Stonard MD. Assessment of nephrotoxicity. In Animal clinical chemistry. A primer for toxicologists, Taylor & Francis Ltd., London: 1996, pp. 87-98.
52. Bush BM. Interpretation of laboratory results for small animal clinicians. Blackwell Scientific Publications, Oxford: 1991.
53. Kaplan MM. Clinical chemistry: Interpretation and Techniques, 2nded, Lea and Febiger Publication, Philadelphia: 1983, pp. 158-60.
54. Wallach J. Interpretation of Diagnostic Tests. 8th ed, Lippincott Williams & Wilkins: 2006, pp. 1200.
55. Marks V, Cantor T, Mesko D et al. Differential Diagnosis by Laboratory Medicine, Springer Verlag: 2003, pp. 1106.
56. Zilva JF, Panmall PR, Mayne PD. Clinical Chemistry in Diagnosis and Treatment, 5thedition, England Clays Ltd., St. Ives Plc., England: 1991.